BACTERIOPHAGE LIBRARY USEFUL FOR TYPING BACTERIA AND SYSTEM AND METHOD UTILIZING SAME

FIELD AND BACKGROUND OF THE INVENTION

The present invention relates to a library of bacteriophages and an array system and method incorporating same which are useful for typing bacteria. More particularly, the present invention relates to a library including wild type and mutant phages, which phages are presented in an array configuration which produces a displayed pattern of infection which is unique to a bacterial type.

Food-borne diseases have a major impact on public health. In the United States alone, food-borne illnesses affect 60 to 80 million persons yearly, causing 9,000 deaths, and costing an estimated 5 billion U.S. dollars. The estimated number of yearly cases of non typhoid Salmonellosis in the U.S. alone is 2 million, which cases result in up to 2,000 deaths annually. The emergence of food borne diseases is attributed to changes in human demographics and behavior, technology, international travel and international commerce. In addition, microbial adaptation and economic development also contribute to the emergence of food borne diseases. Non typhoid Salmonellosis is one of the most prevalent among food borne diseases in the United States. In the last two decades, the World wide increase in Salmonellosis cases is thought to be linked, at least in part, to centralized food production and large-scale food distribution practices adopted and practiced by modern food industries.

In Israel, for example, over the last 10 years, the number of disease cases caused by *Salmonella* per year has been approximately 5,000. The total positive diagnosis of *Salmonella* contaminated reservoirs (farm animals and birds, human, food and environment) is 10,000 per year. Of the 2,400 existing serovars of *Salmonella*,

approximately 100 belong to the pathogenic non typhoidal bacteria. Of these 100 pathogens, 35 contribute to more than 98 % of all *Salmonella* related disease cases. Of these 35, six serovars (i.e., *S. enteritidis, S. typhimurium, S. hadar , S. virchow, S. infantis* and *S. agona)* cause more than 70 % of all *Salmonella* related disease cases. Ninety percent of all serotyping of *Salmonella* in Israel is performed in the Central Diagnostic Laboratory of the Ministry of Health in Jerusalem. The test is expensive and takes a few days to complete.

Although typing and management of infected sources is exercised in modern countries, the persistence of pathogenic bacteria reflects the difficulties involved in properly understanding the complex interactions between bacteria, environment, and susceptible host populations.

Methods currently employed in classifying (typing) bacteria are subject to a great deal of controversy since bacterial pathogenicity, which in some bacteria is generated by mechanisms yet to be resolved, is oftentimes difficult to describe and/or type.

The clinical classification of bacteria, is typically divided into three categories: systematics, phenotypic characterization, and pathogenicity.

Bacterial systematics is the discipline that deals with identification and grouping of bacteria into groups. It employs three tools: (i) classification - the arrangement of bacteria into groups according to genetic characteristics; (ii) nomenclature - the naming of bacteria, according to internationally accepted standards; and (iii) identification - the comparison of unknown bacteria with already classified bacterial standards on the basis of phenotypic/genetic characteristics.

These tools allow classification of the various kinds of bacteria and the comparison of unknown organisms to bacteria that have already been classified (bacterial standards).

The basic unit for classification in bacteriology is the species. A species in bacteriology consists of a type strain, grouped together with all other strains that are sufficiently similar to that type strain. The type strain is a strain that has been designated as the standard example of that species. All other strains being considered for inclusion in a species must be compared with the type strain of that species. Cultures of type strains can be purchased from various reference collections. Qualitative characteristics of phenotypic properties, such as morphology, structure, cultivation, nutrition, biochemistry, metabolism, pathogenicity, antigenic properties, and ecology are used in traditional and routine classification tests. A relationship between bacterial isolates can also be tested on the genetic level. The great advantage of bacterial genetic classification (phylogenetic) is that, since it depends on factors which are stable, the resultant classification is not subject to constant change. Nowadays, genetic classification of bacteria, in most cases, relies on sequence comparison of ribosomal DNA (encoding ribosomal RNA, rRNA).

Thus, phylogenetic relationships among bacteria can be deduced from analysis of bacterial DNA. DNA hybridization and sequencing techniques allow comparison of the entire genome of different bacterial strains. This helps to resolve many taxonomic problems, since it is assumed that bacterial isolates having significantly different DNA base composition do not belong to the same species. DNA hybridization techniques allow comparison of the entire genome of one bacterial isolate with that of other isolates or standards on the basis of nucleotide base sequence. DNA hybridization is most useful at the species level of classification. A species classification based on DNA hybridization can usually be readily defined in phenotypic terms, because the strains in the species tend to be very similar to one another not only in genotype but also in phenotype. However, above (genus and higher) and below

(strains) the species level, the genotype/phenotype correlation is often difficult to observe.

Unlike eukaryotes in which phenotypic characterization is based mainly on morphological variations, prokaryotic (bacteria) phenotypic characterization is based on molecular variations. The commonly used techniques for bacterial typing include serological analysis, biochemical analysis, genetic analysis and bacteriophage typing.

The typing of pathogenic bacteria on the other hand is a process based solely on phenotypic analysis. The assignment of a name to a bacterial isolate is done according to a best fit with a bacterial type standard.

Several different phenotypic characterization techniques are traditionally utilized to characterize pathogenic bacteria, including bacterial enzymatic activities, surface receptors recognition, surface antigens recognition and bacteriophage infectability (bacteriophage typing). A well calibrated system provides very reliable results. However, reliable results are often difficult to attain under routine conditions since results from control tests which can indicate false reactions are often unreliable. In addition, cross reactivity of bacterial surface molecules and poor enzymatic activities in test conditions produce many false results and reduce diagnostic reliability. To overcome such problems, the diagnostic process is performed in several steps using different techniques for each diagnostic test. A semi-accurate identification of the bacterial species can also be provided by the colony morphology when grown on selective solid growth media chosen on the basis of reactivity of the bacteria to selective properties of the growth nutrient. Identification of the bacterial genus and species also requires a standard profile which is generated from activities of several enzymes (e.g., API).

The most common method of characterizing pathogenic bacteria involves surface marker typing. This procedure is traditionally performed by an immuno-assay. Many commercial kits are available to this end, which employ monoclonal, polyclonal, or monospecific (e.g., affinity purified) antibodies. In immuno-assays the diagnostic procedure is divided into several consecutive steps. The results can provide a definite antigenic analysis only following a very laborious procedure. In most cases, the antigenic analysis tests the presence of a small number of surface constituents. In the case of monoclonal antibodies it relates to only a single immunogenic determinant (epitope). In some bacterial systems antibodies do not provide sufficient differential diagnosis for subclassing of bacteria and, therefore, other marker systems must be used. For example, the *Salmonella enterica* serovars *S. typhi, S. typhimurium, and S. enteritidis* are classified by immune sera but are additionally subdivided by bacteriophage typing sets.

Bacteriophages (commonly called phages) are bacteria infecting viruses which display host specificity. During a course of infection, bacteriophages gain access to the host bacterium via bacterial cell surface constituents (referred to as receptors) through specific recognition and attachment interactions between these receptors and bacteriophage particle surface ligands.

The concept of using bacteriophages to type host bacteria is commonly practiced in the art although such a typing method is typically utilized to further complement serotyping and other pathogenic typing methods. Many authors have disclosed how the specificity of bacteriophages may be used to distinguish between bacterial genus, species or serotype (serovar). In J. Clin. Microbiol. 20 (1984) 1122-1125, Cooper *et al.* disclose the use of bacteriophages to distinguish between certain species of Bacteroides. Van der Walt and Stein disclose

how Salmonella and *Citrobacter* sub-species may be differentiated using the specificity of bacteriophages [Onderstepoort J. Vet. Res. 56 (1989) 263-269]. He and Pan, in J. Clin. Microbiol. 30 (1992) 590-594, disclose how bacteriophages may be used to distinguish between types of *Enterobacteria* present in clinical specimens, and Liew and Alvarez demonstrated that subtypes of *Xanthomonas campestris* can be distinguished using the specificity of bacteriophages [Phytopathol. 71 (1981) 274-276].

Detection of specific bacteria via genetically engineered bioluminescent bacteriophages which have had the 'lux' gene inserted into their genome has also been described [Ulitzer and Kuhn, in Scholmerich *et al* (Eds) "Bioluminescence and chemiluminescence - new perspectives", pages 463-472: published in 1987 by John Wiley and Sons]. This technique is based on the fact that upon infection of a target bacterium, bacteriophage genes and the lux gene are injected into the host bacterium and are subsequently expressed. The presence of a target bacterium is indicated by emission of light generated from the activity of the 'lux' gene which can easily be measured. Most bacteria are susceptible to attack by bacteriophages, many of which lyse or disrupt their host at the end of their replication process, and these interactions show varying degrees of host/phage specificity. Schutzbank *et al.* have shown the potential of this technique but note problems with cross reactivity between construct phages and other non-target bacterial types. While such problems may be overcome by engineering more specific phages, see, for example, U.S. Pat. No. 4,348,478, this entails provision of phages for each type of target bacteria for which a need to test exists. Such recombinants may not readily be constructed for a variety of reasons, not least of which being the need to avoid disruption of the function of the phage itself. In addition, to screen and differentiate a

large number of bacterial types a large number of phages must be employed, since each is dedicated to the typing of a single bacterium.

To overcome the problems associated with the use of recombinant phages and yet provide an efficient method for the detection of bacteria using wild type phages, U.S. Pat. No. 5,888,725 to Sanders describes a method for detection, identification and/or quantification of target organisms of specific bacterial genus, species or serotype, based upon the occurrence of release of cell contents, particularly nucleotides, e.g., ATP, upon lysis of bacteria by bacteriophages (phages) specific for them.

Although bacteriophages can be utilized for bacterial typing currently employed bacteriophage typing methods cannot be used as a sole classification method because of the limited number of phages available for such classification.

As such the methods described in the prior art documents mentioned hereinabove are typically dedicated to the typing of a narrow range of bacteria and in general are only useful for the detection of the presence of a particular bacteria. As such, these prior art methods cannot be utilized to differentiate between closely related bacterial types for which differential infecting bacteriophages do not exist. In addition these methods cannot be used to type a wide range of bacteria since they are limited by the phage types available.

There is thus a widely recognized need for, and it would be highly advantageous to have, a bacteriophage mediated bacterial typing method devoid of the above mentioned limitations which are inherent to prior art bacteriophage typing methods. Specifically, there is a widely recognized need for, and it would be highly advantageous to have, a bacteriophage mediated bacterial typing method which is self sustained in that it is sufficiently efficient for genus, species, strain, serovar and pathogenicity typing. There is also a widely recognized need for, and it would be

highly advantageous to have, a method of producing a plurality of phage mutants useful while implementing bacteriophage mediated bacterial typing method.

SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided a bacteriophage library useful for typing bacteria, the bacteriophage library comprising a plurality of bacteriophages being categorized into (a) a first category including bacteriophages being infective to a first type of bacteria; (b) a second category including bacteriophages being infective to a second type of bacteria; and (c) a third category including bacteriophages being infective to both the first type and the second type of bacteria.

According to another aspect of the present invention there is provided a method of typing bacteria present in a sample, the method comprising the steps of (a) incubating the sample with an arrayed library of bacteriophages being categorized into (i) a first category including bacteriophages being infective to a first type of bacteria; (ii) a second category including bacteriophages being infective to a second type of bacteria; and (iii) a third category including bacteriophages being infective to both the first type and the second type of bacteria; and (b) identifying bacteriophages being infective to at least one bacteria in the sample; and (c) correlating between an identity of the bacteriophages being infective to the at least one bacteria and an identity of bacteriophages of the library known to be infective to bacterial standards, so as to enable typing of the at least one bacteria present in the sample.

According to further features in preferred embodiments of the invention described below, the step of incubating the sample with the library of bacteriophages is performed in a presence, or with subsequent

addition of, an assay reagent for identifying presence or absence of infection between any specific bacteriophage of the library and bacteria in the sample.

According to still further features in the described preferred embodiments the assay reagent is a polynucleotide intercalating agent selected from the group consisting of ethidium bromide and propidium iodide.

According to still further features in the described preferred embodiments the step of incubating the sample with the library of bacteriophages is carried out on or in a medium supporting bacterial growth.

According to still further features in the described preferred embodiments the medium is selected from the group consisting of a solid medium and a liquid medium.

According to still further features in the described preferred embodiments the bacteriophage library is provided as a preparation selected from the group consisting of a plurality of individual bacteriophage suspensions, a plurality of freeze dried individual bacteriophage powders and a solid support carrying a plurality of individual bacteriophages.

According to yet another aspect of the present invention there is provided a system for typing bacteria present in a sample, the system comprising (a) a library of bacteriophages being categorized into (i) a first category including bacteriophages being infective to a first type of bacteria; (ii) a second category including bacteriophages being infective to a second type of bacteria; and (iii) a third category including bacteriophages being infective to both the first type and the second type of bacteria; and (b) a detector being for detecting a presence or absence

of infection between at least one bacteria in the sample and individual bacteriophages of the library.

According to further features in preferred embodiments of the invention described below, the system further comprising a processing unit being for comparing the presence or absence of infection as detected by the detector to a presence or absence of infection between bacteriophages of the library and known bacterial standards, so as to enable typing of the at least one bacteria.

According to still further features in the described preferred embodiments the system further comprising a processing unit being for comparing the presence or absence of infection as detected by the detector to a presence or absence of infection between bacteriophages of the library and known bacterial standards, so as to enable typing of the at least one bacteria.

According to still further features in the described preferred embodiments the library is provided as an array such that each of the plurality of bacteriophages occupies a specific location of the array.

According to still further features in the described preferred embodiments the bacteriophages of the array are each provided in a liquid medium.

According to still further features in the described preferred embodiments the liquid medium is capable of supporting bacterial growth.

According to still further features in the described preferred embodiments the bacteriophages of the array are each attached to a solid support.

According to still further features in the described preferred embodiments the solid support is selected from the group consisting of a membrane, an agar surface, a microtiter plate, beads and optic fibers.

According to still further features in the described preferred embodiments the detector is capable of visually detecting plaques.

According to still further features in the described preferred embodiments the detector is capable of detecting a presence of released bacterial constituent associated with bacterial lysis.

According to still another aspect of the present invention there is provided a method of uncovering mutant bacteriophages useful in typing bacteria, the method comprising the steps of (a) providing a sample of bacteriophages at a first routine titer dilution; (b) concentrating the sample of bacteriophages to a second routine titer dilution, the second routine titer dilution being more concentrated than the first routine titer dilution; (c) infecting a first bacterial sample with the sample of bacteriophages from step (a); (d) infecting a second bacterial sample identical to the first bacterial sample with the sample of bacteriophages resultant from step (b); and (e) only if the second bacterial sample is lysed, whereas the first bacterial sample is not, isolating bacteriophages from the second bacterial sample, thereby uncovering mutant bacteriophages useful in typing bacteria of the bacterial samples.

According to an additional aspect of the present invention there is provided an array of bacteriophages useful for typing bacteria, the array comprising a plurality of distinct bacteriophages each occupying a distinct location of the array, at least a portion of the plurality of distinct bacteriophages being capable of infecting more than one bacterial host type.

According to still further features in the described preferred embodiments the plurality of distinct bacteriophages are attached to a solid support.

According to yet an additional aspect of the present invention there is provided a method of typing bacteria, the method comprising the

steps of (a) providing an array of bacteriophages including a plurality of distinct bacteriophages each occupying a distinct location of the array, at least a portion of the plurality of distinct bacteriophages capable of infecting more than one bacterial host type; (b) reacting the array of bacteriophages with a bacterial sample so as to produce a first pattern of bacterial plaques on the array; and (c) comparing the first pattern to patterns of bacterial plaques resultant from reacting the array of bacteriophages with known bacterial samples, so as to enable typing of the bacterial sample.

According to further features in preferred embodiments of the invention described below, the library is provided as an array, such that each of the plurality of bacteriophages occupies a specific location of the array.

According to still further features in the described preferred embodiments the plurality bacteriophages of the array are each provided in a liquid medium.

According to still further features in the described preferred embodiments the liquid medium is capable of supporting bacterial growth.

According to still further features in the described preferred embodiments the plurality of bacteriophages of the array are each attached to a solid support.

According to still further features in the described preferred embodiments the solid support is selected from the group consisting of a membrane, an agar surface, a microtiter plate, beads and optic fibers.

According to still further features in the described preferred embodiments the library includes mutants of known bacteriophages the mutants being characterized by bacterial host specificity different than the known bacteriophages.

According to still further features in the described preferred embodiments the first type and the second type of bacteria are each bacteria responsible for a food borne disease.

According to still further features in the described preferred embodiments the first type and the second type of bacteria are each of a bacterial genus selected from the group consisting of *Salmonella*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Listeria*, *Campylicbacter*, *Klebsiella*, *Yersinia*, *Pseudomonas* and *Escherichia*.

According to still further features in the described preferred embodiments the first and the second bacteria types are different bacterial species of the same genus.

According to still further features in the described preferred embodiments the first and the second bacteria types are different bacterial strains of the same species.

According to still further features in the described preferred embodiments the first and the second bacteria types are different bacterial serovars of the same strain.

According to still further features in the described preferred embodiments each of the first, second and third categories include N bacteriophages, whereas N is an integer selected from the group consisting of integers between and including 2 and 10,000.

According to still further features in the described preferred embodiments the library is sufficiently diversified bacteriophage content so as to enable the typing of all known constituents of a bacterial genus.

The present invention successfully addresses the shortcomings of the presently known configurations by providing a bacteriophage mediated bacterial typing method which is self sustained in that it is sufficiently efficient for genus, species, strain, serovar and pathogenicity typing. The present invention further successfully addresses the

shortcomings of the presently known configurations by providing a method of producing a plurality of phage mutants useful while implementing bacteriophage mediated bacterial typing method.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 is a schematic depiction of a system for typing bacteria according to the teachings of the present invention.

FIGs. 2a-d are photographs of agar plates including bacteriophage library constituents incubated with *Salmonella virchow* bacteria, showing sites of lysis as detected by a plaque assay with each site corresponding to a different phage type of the library utilized. Figures a-d represent four different sets of the *S. virchow* bacteriophage library developed according to the teachings of the present invention.

FIGs. 3a-d are photographs of agar plates including bacteriophage library constituents incubated with *Salmonella infantis* bacteria, showing sites of lysis as detected by propidium iodide fluorescence with each site

corresponding to a different phage type of the library utilized. Figures a-d represent four different sets of the *S. infantis* bacteriophage library developed according to the teachings of the present invention.

FIG. 4 is a graph depicting the relationship between fluorescence and the number of bacterial cells infected and lysed while implementing the method of the present invention.

FIGs. 5 and 6 are photographs of the prior art API-STAPH typing method showing the various color markers associated with a positive identification of various strains of Staphylococci bacteria. Each strip corresponds to 7 sets of three (6 sets) and two (1 set) marker groups.

FIG. 7 is a table showing the bacteriophage markers of six different isolates (serovars) of *Staphylococcus aureus* (API profile of 6736153). Red numbers represent the phages positive for all serovars. Black numbers represent the phages infective to only part of the serovars. Bold capital letters represent a specific phage set while the numbers represent phage constituents of the set.

FIG. 8 is a table showing positive bacteriophages profiles for various *S. aureus* serovars isolated from infected bovine udders. Bacterial phage markers unique to 6736152 are marked with blue numbers. Bacterial phage markers unique to bacteria 6737150 are marked with green numbers. Red and black phage marker numbers represent phages infective to bacterial strains 6736153 and 6736151. The pink numbers represent phages infective to strains 6736150 and 6736152.

FIG. 9 is a table showing the infectivity of a Staphylococci bacteria phage library. The left column represents propagating Staphylococci strains originating from the international phage typing. The right column represents the marker numbers of phages infective to these strains of Staphylococci.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of an array, system and method incorporating a plurality of bacteriophage types which can be used to type bacteria. Specifically, the present invention can be used to type a wide range of bacteria, say, all know bacterial constituent of a genus harboring several thousands bacteria species, strains and serovars of varying degree of pathogenicity, by providing a diversified array of bacteriophages including mutant bacteriophages, which array can be used to type the wide range of bacteria by correlating a specific array infection pattern characterizing a tested bacteria with standard patterns of known bacteria.

The principles and operation of bacterial typing according to the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

As used herein, the term "type" when used in context of bacteria is meant to include either a genus, a species and a subspecies e.g., a strain and/or a serovar. As such bacteria of a certain type can be bacteria of a certain genus, species or subspecies (strain or serovar). When used in context of bacteriophages, the term "type" refers to the host specificity of that bacteriophage, i.e., bacteriophages of different types have somewhat different bacterial type specificity.

The terms "serovar" and "serotype" are used herein interchangeably and refer to a bacterial isolate which is reactive to specific antiserum prepared against this isolate (polyclonal, mono-specific or monoclonal). As such, a serovar displays unique antigenic determinant(s), some or all of which may or may not be present on other isolates of a strain or on other strains of a species.

Bacteria-phage interactions are substantially more complex than bacteria-antisera interactions, oftentimes involving several, say 2-4, distinct sites of recognition and attachment, each being equivalent in size to an epitope. Therefore, phage-typing can theoretically be employed to replace serotyping and other typing methods, provided sufficient phage diversity in terms of bacterial type specificity are available.

The terms "bacteriophage" and "phage" are interchangeably used herein.

As used herein the term "infection" and "infective" refer to the process in which a bacteriophage attaches to, and enters into a host bacteria. Infection can follow either a lytic path in which the bacteriophage propagates within the host leading to host cell lysis, or a lysogenic path in which integration of the bacteriophage genome into the host genome occurs with no initial bacteriophage propagation and cell lysis. Preferably the term infection is used herein to refer to a lytic infection.

According to one aspect of the present invention there is provided a bacteriophage library useful for typing bacteria. The bacteriophage library according to this aspect of the present invention includes a plurality of bacteriophages, which are divided into at least three categories. A first category includes bacteriophages which are infective to a first type of bacteria. That is to say, that each of the bacteriophage members of this category infects a single specific type of bacteria. For

example, the bacteriophages of this category can infect a certain bacterial species.

The bacteriophage library further includes a second category of bacteriophages which includes bacteriophages infective to a second type of bacteria. This category is similar to the first category with the exception that the bacteriophage constituents of this category infect a different bacterial type, for example, a bacterial species different than that infected by the bacteriophages of the first category.

The bacteriophage library according to this aspect of the present invention further includes a third category of bacteriophages which includes bacteriophages infective to both the first type and the second type of bacteria. It will be appreciated that the bacteriophages of the third category are distinct from the bacteriophages of the first and second categories.

According to one preferred embodiment of the present invention, the first type and the second type of bacteria are each bacteria responsible for a food borne disease. Examples of bacterial genera including such bacterial species include, but are not limited to, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Listeria*, *Campylicbacter*, *Klebsiella*, *Yersinia*, *Pseudomonas* and *Escherichia*.

According to another preferred embodiment of the present invention, the first and the second bacteria types are different bacteria species of the same genus.

According to yet another preferred embodiment of the present invention, the first and the second bacteria types are different bacterial strains of the same species.

According to still another preferred embodiment of the present invention, the first and the second bacteria types are different bacterial serovars of the same strain.

The library of this aspect of the present invention enables the typing of bacteria based on the detection of an infection of bacteria present in a sample with the various library phage constituents. For example, a bacterial isolate which is infected by members of the first and third categories of phages is typed as a first type, while a bacterial isolate infected by members of the second and third categories is typed as a second type. Since the bacteriophage members of this library display varied levels of host cell infectivity, a degree of infectivity, as measured by for example, a degree of lysis can also be used as a typing measure. It will be appreciated that this is the most basic configuration of the library according to the present invention.

Alternatively and preferably the library of the present invention includes numerous categories of either bacteriophages infective to one type of bacteria, or infective to more than one type of bacteria. It will be appreciated that since single serovar infective bacteriophages exist as well as single species infective bacteriophages, a library of numerous categories can be constructed including categories of bacteriophage members which are infective to numerous bacterial types along with categories of bacteriophage members which are infective to a single bacterial type. Such libraries are exemplified hereinbelow in Examples 3, 4 and 6 of the Examples section. As exemplified therein any number of categories including any number of bacteriophages can be used to effect bacterial typing. It will be appreciated that the number of bacteriophage categories and the number thereof utilized in a given library depends on the desired level of typing and the diversity of the bacterial genus to be typed.

It will be appreciated that in order for the library of the present invention to be effective in typing bacteria, the presence or absence of infection must be detected for each bacteriophage member

independently. As such, and according to preferred embodiments of the present invention the library is provided as an array, such that each of the plurality of bacteriophages occupies a specific location of the array. Such an array can be provided on a solid support, such as, for example, a membrane, an agar plate or a microtiter plate, in which cases each bacteriophage member of the library is attached or adhered thereto in a specific predetermined position. In the case of agar plates, such plates preferably include bacterial growth media so as to support bacterial growth. When the array is provided on a membrane, such as, for example, a nitrocellulose or a nylon membrane, in which case a bacterial culture is applied onto the membrane and the membrane soaked with nutrient growth media. In addition, the bacteriophage members can also be provided on beads, in which case a single bacteriophage member can be adhered to a single bead. Alternatively the bacteriophage members can each be provided on an end of an optic fiber, in which case the fiber is used to optically communicate ultra violet light radiation from a light source.

Alternatively, each bacteriophage member of the library can be provided as a suspension in, for example, a well of a microtiter plate. Preferably, the suspension includes a bacterial growth media so as to allow bacterial growth therein.

It will be appreciated that in any case the bacteriophages can be provided either as a pure stock, freeze dried, suspension or the like or as bacterial stock infected with the bacteriophage which can be lysed when needed.

Thus, the library of the present invention can be used to type bacteria as follows. A sample of an unknown bacterial isolate is incubated with the library array. Infection is monitored (and optionally quantified) at each location of the array. A pattern of infections

(presence and absence) is then determined and is compared and matched with patterns obtained from incubating the library of the present invention with known bacterial standards which were typed with prior art methods such as, for example, serotyping.

It will be appreciated that any method suitable for identifying infection can be used by the present invention. For example, infection can be detected by the formation of a plaque resultant from bacterial lysis.

Alternatively and preferably, infection can be detected via the detection of bacterial lysis by-products, such as, for example, intracellular bacterial polypeptides or polynucleotides which are released from the cell following lysis. For example, the appearance and quantity of polynucleotides can be visualized using intercalating agents which uniquely fluoresce following activation upon intercalation into nucleic acids, such as, but not limited to, ethidium bromide, propidium and other membrane impermeable intercalating agents, which are provided either during or following the infection reaction and which are induced to fluoresce using ultraviolet radiation. It will be appreciated that only membrane impermeable intercalating agents can be used by the present invention such that detection of bacterial polynucleotides by the intercalating agent is correlated to bacterial membrane disruption caused by lysis.

As shown in Figure 1, to enable real time detection and processing of bacterial lysis reactions, and as such bacterial typing, the library of the present invention is incorporated into a system for typing bacteria, which system is referred to hereinbelow as system **10**.

System **10** includes a library array **12** which can be provided in any of the forms described hereinabove. System **10** can also include mechanisms enabling automatic provision of a bacterial sample to array

12. For example, if library array 12 is a microtiter plate then system 10 can include a microtiter plate dispenser such that a sample of the isolate to be typed can be provided to each well of the microtiter plate.

System 10 further includes an optical scanning device 14 which serves to collect optical information from array 12. Such optical information can pertain to the appearance and/or size of plaques. Alternatively and preferably such information includes fluorescence collected from array 12, which fluorescence is generated from an intercalating agent which is added to array 12 prior to or following lysis. To generate such fluorescence array 12 is irradiated with ultraviolet light generated from a light source 16.

The pattern of lysis detected and collected by device 14 can then be manually compared to patterns obtained from known, previously typed, bacteria. Alternatively and preferably system 10 includes a processing unit 18 communicating with device 14. Unit 18 serves to automatically compare the pattern obtained by device 14 with a plurality of patterns stored therein to obtain a matching pattern such that typing of the isolate can be effected.

Utilizing a library which includes categories of bacteriophages some of which are infective to several bacterial types significantly decreases the number of individual bacteriophages which must be utilized by this library to effect accurate typing of a wide range of bacteria, say the entire known bacterial types of a bacterial genus. Since typing according to the present invention depends on both the presence and absence of infection in several bacteriophage categories, optionally also combined with a level of infection, a specific pattern of infection can be yielded for each bacteria typeable by this library.

Thus, the library of the present invention is configured such that the bacteriophages diversity thereof is sufficiently high so as to enable

typing of, for example, an entire bacterial species or genus, while, at the same time, by utilizing multi-host specific bacteriophage members, the number of library constituents is kept to a convenient minimum, significantly simplifying the preparation and utilization of such a library.

A major inherent limitation to prior art bacteriophage typing methods arises from the limited bacteriophages available for typing. As such, prior art typing methods can only be used to type a very limited range of bacteria.

Although the configuration of the library of the present invention presents significant improvement in typing range over prior art methods it is still limited by the bacteriophage types available. To enable typing of a wide range of bacteria, novel bacteriophages with novel host specificities must be uncovered.

Thus, according to another aspect of the present invention there is provided a method of uncovering mutant bacteriophages useful in typing bacteria. The method according to this aspect of the present invention is effected by first providing a sample of bacteriophages at a first routine titer dilution (RTD). A typical RTD ranges from 1 to 3 units. This sample of bacteriophages is propagated in a first bacterial type which is an infectable host for these bacteriophages. The sample is then concentrated to a second RTD higher than that of the first RTD, say above 5 RTD. This enables concentrating any mutants present within the sample to a concentration which enables initiation of infection. Two identical bacterial samples which are not normally infected by the bacteriophages above are then incubated with the concentrated and non-concentrated bacteriophage samples. Only if the bacterial sample incubated with the concentrated bacteriophage sample is infected and lysed, whereas the bacterial sample incubated with the non-concentrated bacteriophage sample is not, than bacteriophages are isolated from the

bacterial sample incubated with the concentrated bacteriophage sample, thereby uncovering mutant bacteriophages useful in typing bacteria of the bacterial samples.

While reducing the present invention to practice, a plurality of bacteriophage mutants with known host specificity were uncovered. These mutants are characterized by different host specificities as is compared to their progenitors. For examples of such mutants and for further detail on the method described in this aspect of the present invention the reader is referred to Examples 3 and 4 of the Examples section.

As such, according to a preferred embodiment of the present invention the bacteriophage library includes mutants of known bacteriophages. These mutants are characterized by bacterial host specificities different than that of the known bacteriophages, their progenitors. Bacteriophage libraries which include mutants uncovered according to the teachings of this aspect of the present invention are further detailed in Examples 4 and 6 of the Examples section.

Antigenic analysis of surface molecules is currently considered one of the most effective methods to type bacteria. Results obtained from analysis of bacterial surface markers are however difficult to interpret due to several limitations imposed upon such methods.

Clonal stability of pathogenic bacteria is an important phenotypic characteristic. This property is oftentimes masked by lateral mobility of pathogenic related molecules such as plasmid DNA. Such molecules when expressed within bacteria may leads to an altered phenotype.

Cross reactivity of surface molecules can also limit antigenic analysis of some pathogenic members of closely related bacteria. The problem of antigenic analysis of pathogenic bacteria is even more pronounced when bacterial regulatory pathways are altered by ecological

pathways thus contributing to inconsistencies in molecular expression and as a result of which inconsistencies in a phenotype of a particular bacteria.

For economic reasons, a small number of markers (mostly one per bacterial type) are used for serotyping of most pathogenic bacteria by currently employed commercial kits. Theoretically co-analysis of multiple antigenic determinant can resolve the problem. However, such marker systems are available for a very limited range of bacteria. One example of a multiple antigenic determinant system is the system designed to type *Salmonella enterica* which was developed because of the economic importance associated with the detection of this bacteria.

The presence of more than 2,000 stable pathogenic variants in *Salmonella enterica* and the clinical importance of this species have led bacteriologists to produce a very large set of standard immune sera to antigens present on these bacteria. For other pathogenic bacteria, however, only several polyclonal antisera are available for typing. False positive or false negative reactions and/or cross reactivity of the immune sera utilized present a serious obstacle to an accurate interpretation of results obtained from these tests. The use of monoclonal antibodies for the detection of surface antigens in diagnostic bacteriology can resolve some of the problems inherent to polyclonal sera. However, the cost of these markers is still high and the signals obtained suffer from essentially the same problem as polyclonal antisera.

The method system and library array of the present invention which are based on the use of bacteriophage libraries in place of antibodies overcome the problems inherent to prior art bacterial typing methods. As demonstrated in Example 6 of the Example section provided hereinbelow, the information obtained by the use of phage library specific for *Salmonella enterica* is as accurate as many mono-

specific prior art anti-sera assays. The advantage of the method of the present invention is that the typing is performed in a single stage taking 1-2 hours instead of the several days the immune sera based test requires.

The data obtained for the diagnosis of *Staphylococci* using a bacteriophage library constructed according to the teachings of the present invention demonstrates that it is possible to differentiate these bacteria on the level of species (the coagulate positive *S. aureus* and coagulate negative *Staphylococci*) or strain. As such, the present invention provides a rapid and accurate method of classification or typing for these bacteria, for which at present no accurate prior art typing methods exist.

In addition, the multiple marker system generated from the use of the phage library of the present invention reveal an ordered pattern of distribution of the bacterial surface phage markers. Even though the above information is obtained from a limited number of bacterial systems, some general assumptions can be made. The two marker categories of *Staphylococcus aureus* which originated from bovine mastitis cases suggests that the surface of these bacteria contain a stable molecular fraction and a non stable molecular fraction. These two categories of markers can explain the existence of subspecies which are unique for each different host, and the existence of pathogenicity. The existence of similar marker systems in *Salmonella hadar* and *Salmonella agona* suggest that this type of molecular surface organization might also exist in other important pathogenic bacteria.

Control tests are very important in bacterial diagnostic kits because of the considerable amount of false reactions that are known to occur. In routine testing, however, controls are usually omitted because of the technical difficulties involved in running these additional tests.

False reactions can be generated by two different causes. The first is due to background signals resulting from the small change in shading at the point of attachment of the antisera to the antigenic marker(s). A detector such as a CCD camera which is typically utilized in such system misinterprets the differences in shading in the area of attachment because pigmentation of the whole diagnostic area by bacteria is not constant and as such the contrast cannot be standardized. A second cause arises from blockage of positive phage markers on the bacteria.

In the system and method of the present invention false reactions are minimized. Since each array is composed of numerous bacteriophages and since a positive result is defined by a pattern of reaction of a portion of these bacteriophage, which portion is determined by the bacteria tested, the system and method of the present invention is provided with intrinsic controls. Thus false reactions are nullified because of the large number of markers tested. The chances for no or low typing is reduced but can be overcome providing the diversity of the library is expanded by, for example, continued development of new bacteriophage types which are useful for bacterial typing.

In addition, since bacterial infection by bacteriophage typically depends on the co-attachment of the phage particle to several bacterial surface determinants, the method of the present invention is more accurate and less prone to false reactions due to unintentional cross reactivity than a method using antisera typing which is dependent in most cases on the identification of only one determinant.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the

claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait,

M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

Solutions and growth media

Nutrient broth: 8 g/l Nutrient base (Difco, USA) + 5g/l NaCl + distilled water sterilized at 121 °C for 20 minutes.

Nutrient agar: Nutrient broth + 15 g/l Bacto agar (Difco, USA) sterilized at 121°C for 20 minutes.

Rich nutrient broth and agar: As above, with the addition of 20 g/l nutrient base.

Agar plates: Agar plates for phage typing are made from rich nutrient agar + 20 ml/l of 2 % $CaCl_2 \cdot 7H_2O$ added to the sterilized agar at a temperature of 50 °C.

Soft Agar: 5 g/l Nutrient agar (Difco, USA) + distilled water and 20 g/l of 2 % $CaCl_2 \cdot 7H_20$.

Brilliant Green Agar: 58 g/l of Brilliant Green Agar (Difco, USA) + distilled water sterilized at 121 °C for 15 minutes.

EXAMPLE 1

Identification of standard bacteria

Standardization of Staphylococci bacteria: Two hundred wild type *Staphylococci* bacteria isolates were collected from mastitis infected milk. Bacteria from contaminated milk samples were grown on blood agar plates. Single hemolytic colonies were identified as *Staphylococcus aureus* according to a positive coagulase test. Coagulase negative suspected *Staphylococci* bacteria were isolated according to their colony morphology on blood agar plates. All bacteria were tested by the API standard test for *Staphylococci* (API-STAPH, bioMerieux, France, http://www.biomerieux-vitek.com/).

API-STAPH test: The API-STAPH test consists of biochemical reactions of a pure tested bacterial culture with 19 substrates. The substrates utilized in this test are: D-glucose, D-Fructose, Maltose, Lactose, D-Trehalose, D-Mannitol, Xylitol, D-Melibiose, Potassium nitrate, β-naphthyl-acid phosphate, Sodium pyruvate, Raffinose, Xylose, Sucrose, α-methyl-D-glucoside, Arginine and Urea. A positive reaction in this test is indicated by a color change of any of the substrates used. The reaction markers are grouped into 7 groups of three markers each. Scores of 0, 1, 2 and 4 are given to the reactions of each of the three markers of the 7 marker groups, a negative reaction rating a "0", while a positive reaction rating "1", "2" or "4" according to marker values. A final bacterial profile consists of a score for each of the 7 groups which is in fact composed of the individual scores of the 3 marker groups. Typical API profiles of 12 *Staphylococcus* strains are shown Figures 5 and 6. The whole profile refers to a specific *Staphylococcus* strain. Typical values for the coagulase positive *Staphylococci* (*Staphylococcus aureus)* are 6736130 to 6736153. *S. sciuri* and *S. xylosus* are closely related to *S. aureus* and they have an API profile of 6736050 and 6736552 respectively. Another *Staphylococci* bacteria present in mastitic

cows is *S. chromogenes*, which is classified as having an API of 6717662. Bacteria having an API value outside the range of *Staphylococcus aureus* are referred to as coagulase negative *Staphylococci*.

The API test is routinely used for the diagnosis of *Staphylococci* bacteria and is accepted as a standard test. The identification obtained is based on the classification of Kloos and Schleifer (see, for example, Kloos and Schleifer, J. Clin. Microbiol. 1:82-88, 1975; Radebold, K., Essers L. Evaluation of the API-Staph Micro-System for Routine Identification of Staphlococci (1980) Arzt. Lab 26, 236-238; Pascoli L., Chiaradia V., Micignat G., Santini G., Identification of Staphylcocci by the API – Staph, (1986) Eur, J. Clin. Microbiol. 5, 6, 669-671).

Salmonella isolates: Wild type *Salmonella* bacteria were collected from contaminated food. All suspected *Salmonella* bacteria were grown on brilliant green agar, a selective media for *Salmonella* (Difco, USA). One thousand *Salmonella* isolates belonging to 35 different serovars were identified using standard agglutination serum for *Salmonella* used according to the protocol of the World Health Organization (WHO) Collaborating Center for Reference Research on *Salmonella* (1997) at the Institute Pasteur, Paris, France. Table 1 below lists the bacterial serovars and the antigenic formula obtained from the diagnosis of the bacteria in this collection.

The 35 types of *Salmonella* isolates have 10 to 100 repeating copies. They include greater than 90 % of the major non-typhoid contaminating *Salmonella* types found in Israel in the last 5 years.

TABLE I

Salmonella enterica serovars typed with a library of phages according to the present invention showing 10 phages unique to each bacterial serovar (under the results column)

Isolate	Name	O Antigen	Phase 1	Phase 2	Results
15	Paratyphi B	-1,4,[5],12	b	1,2	A(5, 15, 6), B(16,25, 27,29, 43), D(7,10)
42	Saintpaul	-1,4,[5],12	e,h	1,2	A20, B(16, ,7,2,17,21, 24,44,15), C4
43	Reading	-1,4,[5],12	e,h	1,5	A(26,36,38,42,49, 58), B(25, ,29,63), D10
51	Agona	-1,4,12	f,g,s	[1,2]	A(42,58,62), C(4,12,41, 48), D(,7,21,31)
66	Typhimurium	-1,4,[5],12	i	1,2	B23, C(18,21,23,24,25, 27,56) ,D(18,20)
85	Brandenburg	-1,4,[5],12,-27	l,v	e,n,z15	C(14,18,21,23,25, 27), D(33,39,54), E4
99	Heidelberg	-1,4,[5],12	r	1,2	A(42,62,5,20), D(10, 33, 47,49) ,E(11,25)
129	Haifa	-1,4,[5],12	z10	1,2	A58, B(20, 24, 25, 43, 44, 61), C(19,43), D10
184	Livingstone	6,7,-14	d	l'w	A(41,49, 62), B(14,28, 44,46, 48,61), E9
197	Montevideo	6,7,-14	g,m,[p],s	[1,2,7]	A14, B(2,9, 22, 23,41, 53), C(62,33,63)
211	Oranienburg	6,7,-14	m,t	[z57]	B(4,6,18,23,41), C(18, 21, 25,30), D54
220	Thompson	6,7,-14	k	1,5	A(3,43,50), B(9,43, 44, 53,61) ,C33, D2
227	Concord	6,7	l,v	1,2	A(20, 26, 32, 41,42, 49, 58), B(25, 30,45)
231	Bonn	6,7	l,v	e,n,x	A(,26,22,32,49,45,58), B(16,30,25), C51
248	Virchow	6,7	r	1,2	B(6,18,23), C(12,62), D(18, 32,34, 54, 63)
249	Infantis	6,7,-14	r	1,5	A43, B(2,9,14,16,20, 22,24,56), C52
286	Jerusalem	6,7,-14	z10	l,w	A(14,15, 26,32,42, 49, 55,58), B25, D28
289	Tennessee	6,7,-14	z29	[1,2,7]	B(4,18,23), C(5, 24,25, 30,40), D39 ,D52,
341	Muenchen	6,8	d	1,2:[z67]	A(32,38), C(13,29,40), D(13,35,36), E(16,18)

TABLE 1 (Continued)

343	Manhattan	6,8	d	1,5	A(26, 32, 38, 41,42, 49, 55,58), B(25,30)
351	Newport	6,8,-20	e,h	1,2:[z67]	C(5,13,17,18,29,42,50), D(13,38,52)
353	Kottbus	6,8	e,h	1,5	A14, C(6, 25,30,56,62), D(54, 44,45), E4
360	Emek	8,-20	g,m,s	-	C(13,17,24,31,32,40,42) D(13,52), E10,
379	Kentucky	8,20	i	z6	A(26,41,58), B25, C(10, 19,51), D(28,56,34)
382	Blockley	6,8	k	1,5	A(5,6,15,10,39), B61, C(11,19), D29, E11
442	Hadar	6,8	z10	e,n,x	B(4,6,18,23), C(5,18,21, 24,25,30)
489	Eastbourne	-1,9,12	e,h	1,5	A(58,20,10), B(20, 24, 25,28,30,45,61)
495	Enteritidis	-1,9,12	g,m	-	C(4,10,11,12,53,55,39, 49), E(3,11)
666	Anatum	3,10[-15][-15,-34]	e,h	1,6	B(4,6,18,3,41,15), C41, D(21,55), E19

The bold letters represent phage set and the numbers represent phage position in the set. Columns marked O antigen, phase 1 and phase 2 represent results from antisera typing.

EXAMPLE 2

Bacteriophages

Selection of bacteriophage sets: A phage set includes up to 30 phages active against a portion of the bacterial stock. Bacteriophages (phages) were isolated from lysogenic bacteria by standard Mitomycin induced release of temperate phages (using Mitomycin C from Sigma Chemicals, St. Louis, Missouri, USA, Cat. No. M-0503). Bacteria were grown overnight in liquid nutrient broth (Difco, USA) and a 200 µl sample of the bacterial suspension was used to inoculate 2 ml of pre-warmed nutrient broth including 2 µg per ml Mitomycin C. The inoculated nutrient broth was incubated for 30 minutes at 37 °C following which it was centrifuged in a microfuge at 14,000 rpm for 10

minutes. The supernatant was collected, a drop of chloroform was added, and the mixture was shaken at 250 rpm at room temperature (21 ° C) for one hour. A 200 µl sample of the supernatant was added to 0.5 ml of bacterial culture and plated on a nutrient agar plate. Excess bacterial fluid was aspirated and the plate was allowed to dry in a laminar flow hood. The plate was then incubated for 18 hours at 37 °C. Single phage infections were demonstrated by the formation of small individual plaques. Each single plaque was collected into 2 ml of the starting bacterial culture and incubated at 37 °C for 18 hours. Propagation was performed by transferring 200 µl of the phage supernatant into 5 ml of soft agar which includes 200 µl of the bacterial culture in nutrient broth. The soft agar was plated on a rich nutrient agar plate and incubated at 37 °C for 18 hours. A pure phage suspension was isolated from the soft agar and incubated in 5 ml of nutrient broth to release the entrapped phages. Following centrifugation, the supernatant was filtered through a 0.45 µm Millipore filter, a drop of chloroform was added and the phage suspension was stored at 4 °C. Determination of phage concentration was achieved by infecting confluent bacterial culture agar plates with serial dilutions of the phage suspension. This enabled to determine the highest dilution which still produces confluent lysis. A routine titer dilution (RTD) is calculated on the basis of the highest lysis producing dilution of a logarithmic series. The results are represented as the log of the actual dilution and were in this case between 3 to 8 RTD.

Propagation, enrichment and determination of phage concentration (RTD): To concentrate a phage suspension of a very low titer, 200 µl of the phage suspension and host bacteria were introduced into 2 ml of a nutrient broth growth media. Following incubation for 18 hours at 37 °C the upper liquid phase of the growth media was separated via centrifugation and filtered through a 0.45 µm Millipore filter. This

resulted in a concentration of phages particles to an RTD value of 4. A 200 µl sample of RTD-4 phages was introduced into 5 ml soft agar filled test tubes which included 100 µl of 2 % Ca^{++}. The tubes were shaken gently and plated on a nutrient agar plate and the plates were incubated at 37 °C for 18 hours. Phages were collected from the soft agar as described hereinabove. The RTD concentration was determined by serial dilutions and calculated according to the highest dilution that still produced a confluent lysis.

Typing of bacteria by phages: A bacterial lawn was prepared on nutrient agar 90 mm Petri dishes. A 3 µl drop of a predetermined phage concentration was applied onto the plates in a pattern of 30 to 60 points which generated a grid or array formation. Following incubation at 37 ° C for 18 hours, plaques were formed at points including matching reactive phages. As determined from these experiments, an optimal typing concentrations for *Salmonella* and *Staphylococci* typing phages are of RTD values of 1 and 2, respectively.

EXAMPLE 3

Mutant phages and phage libraries

Isolating phages mutants: Naturally occurring phage mutants are found in very low titers in pure phage suspensions. Since a threshold concentration is needed in order to initiate infection, phage suspensions including suitable infecting mutants must be concentrated above an RTD value of 5-8.

Bacteria are typed with a working solution of phages (RTD of 1-2 for *Salmonella* and RTD of 1-3 for *Staphylococci*). Typing is effected as described in Examples 1 and 2. Typing is performed for each bacteria with both the concentrated phage suspension (above RTD of 5-8) and an optimal typing concentration (RTD of 1-3). Typing at the low and high

concentrations reveal positively matching bacteria and phages. When a positive typing reaction is achieved only in the more concentrated phage suspension it is indicative of the presence of mutant phages. The mutant phages are enriched and propagated as previously described.

Production of mutant phage libraries: Phage libraries were prepared for *Staphylococci* and non-typhoid *Salmonella enterica*. In both cases the library includes approximately 1,000 phage mutants divided into 6 sets, each reactive against a stock bacterial species. The generation of these sets was performed in 3 steps. Initially, approximately 60 phage variants were collected from the natural environment of the bacteria. Following the collection, a very large number of mutant phages active against a collection of approximately 500 wild type bacteria were generated (sets 2-5). Finally, a 6th set which was designed to react with 1,000 to 5,000 bacteria representing bacteria from various geographical sources was generated.

The *Staphylococci* initial set contained 30 phages from standard phage typing sets (Colingdale, London, England) which was acquired from the bacteriological laboratory of Asaph-Harophe hospital in Israel. In addition, 30 new phages were isolated from lysogenic bacteria (phages presented in the bacterial genome) by the standard Mitomycin induced phage-releasing method (see Example 1). Bacteria originating from bovine mastitis cases were identified in the Mastitis central laboratory in Saesarea , Israel.

The initial phage set for *Salmonella* contained 12 phages from the international standard phage typing set (Colingdale, London). The bacteriophages include the phage set for *Salmonella enteritidis*: SE-1, SE-11, SE-17, SE-22, SE-34, SE-37, SE-42, SE-44, and the phage set for *Salmonella typhymurium*: ST-1, ST-1a, ST-2, ST-2a, ST-2c, ST-B, ST-C, ST-H, ST-G, ST-F, IO.

These phage sets were acquired from the Central laboratories, of the Ministry of health, Jerusalem, Israel. An additional 48 phages were isolated by Mitomycin induced phage release from lysogenic wild types bacteria. The *Salmonella* bacteria were received from the central laboratory for food control of the Kimron Veterinary Institute, Beit-Dagan, Israel.

Immobilization of phage marker on a solid support: Phage concentrations were adjusted to an RTD value of 3. A 0.5 µl aliquot of each of the various phage strains was spotted onto a nitrocellulose membrane (pore size of 0.2 µm, Millipore) to form a marked grid pattern similar in size, spacing and pattern to 96 well microtiter plates. A device which contains a 4 x 4 array of pins spaced so as to match a portion of a microtiter plate was fabricated. This device was used to transfer phage stocks from the culture plates onto a sterile filter membrane by applying 16 phage strains onto the membrane at a time. This coordinate pattern of phages matches the pattern of wells in microtiter plates, such that when the nitrocellulose membrane immobilized phages are applied to a microtiter plate which contains bacteria, each phage spot is matched to a well.

In order to maintain the immobilized phage viable, following transfer and immobilization, the membranes were coated with a 1 % alginate gel. In addition the membrane was placed on a Wattman # 1 filer paper soaked with sterile double distilled water (ddw) and sealed with a plastic wrap.

Detection of the interaction between bacteria and a positive phage: The interaction between bacteria and positive phages (infection) can be detected in suspension or on solid phase. When bacteria are lysed by a phage, bacterial nucleic acids are released and are detectable and quantifiable by, for example, interaction with an intercalating agent,

propidium iodide in this case, which is not penetrating into intact cells. Bacterial lysis can be visualized by providing propidium iodide in the growth media, such that fluorescence is generated when a lysed sample is illuminated by ultra violet light. Released bacterial nucleic acids are present in detectable quantities after two hours of incubation with a concentrated phage stock.

EXAMPLE 4

Phage typing of salmonella bacteria using the novel mutant phage library

The phage library that was developed for the typing of the *Salmonella virchow* bacterial collection consists of 5 sets, each set containing 60 different phage types (a total of 300 different phage types). The sets are marked with Roman numerals on the plates (Figures 2a-d, 3a-d, one set is not shown) and with the letters "A" to "E" in the tabulated data. In each of the five sets, the phage numbers run from 2 to 63. The phage printing device described above uses a standard 96 well ELISA plate as a source for distribution of the different phage types. Reading of results was performed by a scanner (Power II Umax, USA) controlled by a personal computer (PC) and standard scanning software. Reading the positive plaques was performed by a dedicated software program written in Visual C++. Figures 2 and 3 show patterns typical of *S. virchow* (248) and *S. infantis* (249), respectively. In these Figures only phage sets 1-4 are shown (set 5 is omitted). A typical database result is shown in Table 2 below. The entire Table (3 parts) represents the results of the typing of a single bacterial species - *S. virchow*. Various bacterial isolates of the variant *S. virchow* are marked in the first row. In the second row, the phage set is represented by the letters A to E. As indicated above, the numbers 2-63 represent phage numbers in each set. The symbols (+) and (-) represent the presence or absence of reactivity for the phage markers

used. Cumulative results for isolates of *S. virchow* (248), *S. infantis* (249) and *S. tennessee* (289) are presented in Table 3, which follows Table 2.

TABLE 2

Results of phage typing for 11 different isolates (2-12) of S. virchow (248)

BI	P	2	3	4	5	6	7	9	10	11	12	13	14	15	16	17	18	19	20	21
2	A	-	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-
2	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
2	C	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+
2	D	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+
2	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	B	-	-	+	-	+	-	-	+	-	-	+	-	-	-	-	+	-	-	-
3	C	-	-	-	+	+	-	+	-	-	+	+	+	+	-	+	+	-	-	+
3	D	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	-	+	-
3	E	+	-	+	+	+	+	-	+	-	+	-	-	+	+	-	+	-	-	-
4	A	-	+	-	+	+	-	-	+	-	-	-	+	+	-	-	-	+	+	+
4	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	D	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+
4	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	A	-	+	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	+	-
5	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
5	D	+	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+
5	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8	A	-	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-
8	B	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	-	-	+
8	C	-	-	-	+	+	+	+	+	-	+	+	+	+	-	+	+	-	-	+
8	D	+	-	-	-	-	-	-	-	-	-	+	+	+	-	-	+	+	+	+
8	E	+	-	+	+	+	+	-	+	-	+	-	-	+	+	-	+	+	-	-
9	A	+	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	+	-	+
9	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
9	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
9	D	+	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+
9	E	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
10	A	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-
10	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
10	C	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+
10	D	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	+	+
10	E	+	-	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-	+	+
11	A	-	+	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	+	-
11	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

11	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
11	D	+	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+
11	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	A	-	+	-	+	+	-	-	+	-	-	-	+	+	-	-	-	-	+	-
12	B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
12	D	+	-	-	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+
12	E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

BI - Bacterial Isolate; P = Phage

TABLE 2 (Continued)

BI	P	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
2	A	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
2	B	+	+	-	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+
2	C	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
2	D	+	-	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
2	E	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
3	A	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	B	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
3	C	+	+	+	+	+	+	-	+	+	+	+	+	-	-	+	-	-	-	-	-	+	-
3	D	+	-	+	-	-	-	-	+	-	+	+	+	+	+	+	-	+	+	+	-	+	+
3	E	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	A	+	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	+	+	+
4	B	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+	+	+	+
4	C	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
4	D	+	-	+	+	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
4	E	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
5	A	+	-	-	-	+	-	-	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+
5	B	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+	+	+	+
5	C	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
5	D	+	-	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
5	E	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
8	A	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
8	B	+	+	-	-	+	-	+	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+
8	C	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+
8	D	+	-	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
8	E	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-
9	B	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	+	-	+	-	+
9	C	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
9	D	+	-	+	+	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
9	E	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
10	A	-	-	-	-	+	-	-	+	-	-	+	-	-	+	+	-	-	-	-	+	+	+
10	B	+	+	+	+	+	+	+	-	+	-	-	-	-	-	+	+	-	+	-	-	-	+
10	C	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+
10	D	+	-	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
10	E	+	+	-	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
11	A	+	-	-	-	+	-	-	-	-	-	+	-	+	+	+	+	+	+	-	+	+	+
11	B	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+	+	+	+

11	C	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
11	D	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
11	E	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-
12	A	+	-	-	-	+	-	-	-	+	-	+	-	+	+	+	+	+	+	-	+	+	+
12	B	+	+	+	+	+	+	+	+	+	-	+	-	-	-	+	+	-	+	+	+	+	+
12	C	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
12	D	+	-	+	+	-	-	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
12	E	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-

BI - Bacterial Isolate; P = Phage

TABLE 2 (Continued)

BI	P	44	45	46	47	48	49	50	51	52	53	54	55	56	58	59	60	61	62	63
2	A	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	+	+	-
2	B	+	+	+	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-
2	C	-	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	-	+	+
2	D	+	+	-	-	-	-	-	+	+	+	+	+	-	+	+	+	+	+	+
2	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3	B	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
3	C	-	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
3	D	+	+	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+
3	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	A	+	+	-	-	-	+	+	-	-	-	-	+	-	+	+	+	+	+	+
4	B	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-
4	C	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
4	D	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
4	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	A	+	+	-	-	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+
5	B	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-
5	C	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
5	D	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
5	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	B	+	-	-	-	-	-	-	-	+	+	-	-	+	-	+	-	+	+	-
8	C	+	+	-	-	-	-	+	-	-	-	+	-	+	-	+	-	-	+	+
8	D	+	+	-	-	-	-	-	+	+	+	+	-	-	-	+	-	-	-	+
8	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	A	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	-
9	B	+	+	+	-	-	-	-	-	+	+	-	-	+	+	+	+	+	+	-
9	C	-	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	-	+	+
9	D	+	+	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
9	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10	A	-	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	+	-
10	B	+	+	+	-	+	-	-	+	+	+	+	-	+	-	+	+	+	+	-
10	C	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	-	+	+
10	D	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
10	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	A	-	+	-	-	+	+	+	-	-	-	-	+	-	+	+	+	+	+	+

11	B	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-
11	C	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	
11	D	-	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
11	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	A	-	+	-	-	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	+
12	B	+	+	+	-	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	-
12	C	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+
12	D	+	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
12	E	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

BI - Bacterial Isolate; P = Phage

TABLE 3

Positive infective phages for three bacterial serovars

BI	Positive Results
248	A3,A5,A6,A10,A14,A15,A19,A20,A21,A22,A26,A32,A34,A35,A36,A37,A38,A39,A41,A42,A43, A44,A45,A49,A50,A55,A58,A59,A60,A61,A62,A63,D2,D5,D7,D10,D13,D14,D15,D16,D17,D18,D19, D20,D21,D22,D24,D25,D27,D28,D29,D31,D32,D33,D34,D35,D36,D37,D38,D39,D40,D41,D42,D43, D44,D45,D46,D51,D52,D53,D54,D55,D56,D58,D59,D60,D61,D62,D63,C2,C3,C4,C5,C6,C7,C9,C10, C11,C12,C13,C14,C15,C16,C17,C18,C19,C21,C22,C23,C24,C25,C26,C27,C28,C29,C30,C31,C32, C33,C35,C36,C37,C38,C39,C40,C41,C42,C43,C45,C46,C47,C48,C49,C50,C51,C52,C53,C54,C55, C56,C59,C60,C62,C63,B2,B3,B4,B5,B6,B7,B9,B10,B11,B12,B13,B14,B15,B16,B17,B18,B19,B20, B21,B22,B23,B24,B25,B26,B27,B28,B29,B30,B32,B36,B37,B39,B40,B41,B42;B43,B44,B45,B46, B48,B49,B51,B52,B53,B54,B56,B58,B59,B60,B61,B62,A30,A52,A53,A54,C44,A2,A4,A9,A13,D26, A29,D23,A48,E2,E12,E13,E14,E15,E16,E20,E21,E22,E23,E25,E26,E28,E30,E3,E4,E5,E6,E7,E9,E10, E11,E17,E18,E19,E27,E29,E31,E24,B31,B33,B34,B38,B47,A12,A27,A51,D6,D9,D30,D47,D50,C20, B35,C34,C58,C61
249	A3,A5,A14,A15,A20,A26,A32,A35,A39,A41,A43,A49,A50,A58,A60,A61,A62,A63,B2,B3,B4,B5, B6,B7,B9,B10,B11,B12,B13,B14,B15,B16,B17,B18,B20,B21,B22,B23,B24,B25,B26,B27,B28,B29, B30,B36,B37,B39,B41,B42,B43,B44,B45,B46,B48,B52,B53,B54,B56,B58,B59,B60,B61,B62,D2,D7, D13,D14,D15,D16,D17,D18,D19,D20,D21,D22,D24,D25,D27,D28,D31,D32,D33,D34,D35,D36,D37, D38,D39,D40,D41,D42,D43,D44,D45,D46,D51,D52,D53,D54,D55,D56,D58,D59,D60,D61,D62,D63, C2,C3,C4,C5,C6,C7,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18,C19,C21,C22,C23,C24,C25,C26, C27,C28,C29,C30,C31,C32,C33,C35,C36,C37,C38,C39,C40,C41,C42,C43,C44,C45,C46,C47,C48, C49,C50,C51,C52,C53,C54,C55,C56,C59,C60,,C63,A6,A10,A22,A37,A55,A42,D29,D5,D10,D26, E2,E3,E4,E5,E6,E7,E9,E10,E11,E12,E13,E14,E15,E16,E17,E18,E19,E20,E21,E22,E23,E24,E25,E26, E27,E28,E29,E30,E56,B19,B31,B32,B33,B34,B35,B38,B40,B47,B49,B50,B51,B63,A12,A13,A30, A34,A36,A38,A44,A45,A48,A51,A53,A54,A59,D9,D11,D30,D47,D48,D50,A27,A40,D4,D6,D12, D23,C20,C34,C58,C61

289 B4,B6,B9,B10,B13,B18,B21,B22,B23,B26,D13,D14,D15,D18,D22,D24,D26,D29,D31,D32,D33,D34, D35,D36,D38,D39,D42,D43,D51,D52,D53,D54,D63,C5,C7,C9,C13,C14,C15,C17,C18,C21,C22,C23, C24,C25,C26,C27,C29,C30,C31,C36,C40,C42,C45,C50,C56,B7,B41,D25,D37,D40,D41,D44,D55, D56,C37,B5,D62,E2,E3,E4,E5,E6,E7,E9,E10,E12,E15,E16,E18,E20,E23,E27,E28,E29,E11,E13,E14, E17,E19,E21,E22,E24,E25,E26,E30,D2,D19,D20,D21,D45,D50,D59,C6,C10,C11,C12,C32,C33,C34 ,C35,C38,C39,C46,C53,C54,C59,C62,C63,A3,A5,A14,A15,A20,A22,A39,A43,B2,B3,B12,B14,B15, B16,B17,B24,B34,B36,B37,B38,B39,B42,B43,B44,B52,B53,B54,B55,B56,B58,B59,B61,B62,D4,D5, D6,D7,D9,D10,D11,D16,D17,D23,D27,D28,D30,D46,D47,D58,D60,D61,C2,C3,C4,C16,C19,C20,C28, C41,C43,C44,C47,C48,C49,C51,C52,C55,C58,C60,C61,A12,A26,A30,A32,A34,A35,A36,A37,A38, A41,A42,A44,A45,A48,A49,A50,A51,A53,A54,A55,A58,A59,A60,A61,A62,B11,B19,B20,B25,B27, B28,B29,B30,B32,B33,B35,B40,B45,B46,B47,B48,B49,B51,B60,B63

BI - Bacterial isolate. Bold represent the best 10 markers. Underline represents group O:7 (C1) markers.

Statistical analysis: Statistical analysis of the results was performed by dedicated software programs. A database sheet was used in conjunction with the following programs in order to determine the effectiveness of each phage set employed.

Threshold analysis: Threshold analysis shows the distribution of positive markers among various isolates which belong to the same bacterial serovar. A typical list of threshold analysis for *S. virchow* (248) is shown in Table 4 below. The threshold column which ranges from 60 % to 100 % represents the percentage of the isolates having a positive reaction (infected by) with a given phage. The phage set is marked by a letter and a number which represent the position of the phage on the plate.

TABLE 4

Typical list resultant from threshold analysis

Threshold	Isolate	Phage	Results
60	248	A	3,10,38,39,59
70	248	A	20,22,26,32,35,36,41,42,49,50,60,61,62
80	248	A	14,43
90	248	A	5
100	248	A	
60	248	B	27,29,30,37,45,48,51,54,58
70	248	B	24,60
80	248	B	9,11,12,14,20,25,41,46

90	248	B	2,3,5,7,15,16,17,21,22,26,28,36,39,43,44,52,53,56,59,61,62
100	248	B	4,6,10,13,18,23
60	248	C	44,51
70	248	C	16,48,49,52,53
80	248	C	2,3,4,10,11,19,33,38,39,41,46,47,55,60
90	248	C	7,35,36,37,40,43,54,59,63
100	248	C	5,6,9,12,13,14,15,17,18,21,22,23,24,25,26,27,29,30,31,32, 42,45,50,56,62
60	248	D	7,29,46
70	248	D	2,16,17,41,56
80	248	D	55,58,60,61,62
90	248	D	19,21,25,31,35,37,38,40,44,59
100	248	D	13,14,15,18,20,22,24,32,33,34,36,39,42,43,45,51,52,53,54,63
60	248	E	11,27
70	248	E	3,9,17,29
80	248	E	13,14,19,20,21,22,25,26,28,30
90	248	E	4,5,6,7,10,18
100	248	E	2,12,15,16,23

Letters represent phage set, and numbers represent phage position in the set. Threshold (left column) is represented in percentages.

Duplicate analysis: Duplicate analysis uncovers all phages which cross react with a given bacterial set. Table 5 below lists these phages. The reactivity of any given phage marker that shares reactivity with other phages is analyzed so as to avoid including similar phages within the same library collection. Duplicate reactivity for the *Salmonella* bacteria was analyzed for a set of 500 isolates from contaminated food in Israel. At 100 % threshold no duplicates were uncovered. As such, it was decided to perform the analysis at a 95 % threshold of identity. Duplicate phage markers were excluded from any of the libraries utilized.

TABLE 5

A list of identically infective phage markers at 95 % threshold

Phage	Identically infective phages
A2	A7,A16,A33,A46,A56
A4	A11,A17,A18,A19,A24,A25,A28,A29,A52
A21	A23
A30	A48

A31	A40
A51	A54
B3	B15,B17
B4	B6,B10,B13,B18
B14	B16
B36	B59
C5	C9,C13,C17,C18,C21,C22,C23,C24,C26,C27,C29,C30,C31, C40,C42,C45,C50,D13,D38,E12
D3	D49
D13	D14,D22,D24,D36,D42,D43,D51,D52
D38	D39,D53
E4	E5,E6,E7,E10,E15,E18
E12	E23
E13	E14,E28

List of all positives: A list of all the positive phage markers which are reactive to isolates of, for example, serovars 248, 249 and 289 (*S. virchow, S. Infantis* and *S. tennessee*, respectively) are shown in Table 3.

The best ten: The best ten phage markers for the 35 *Salmonella enterica* serovars are listed in Table 1. The bacterial numbers refer to the serial number published in the 1997 edition of the official publication of WHO collaborating Center for Reference and Research on *Salmonella,* Pasteur Institute, Paris France. The best ten phage markers, for a given bacteria serovar, are selected according to their cross reactivity with the specific serovar but also according to their cross reactivity with other serovars. The best ten phages for the bacteria *S. virchow* and *S. infantis* are marked in bold in Table 3.

Group analysis Group analysis shows phage markers presented only in all members of a given bacterial group. The bacteria 248, 249 and 289 (*S. virchow, S. infantis* and *S. tenesee* respectively) all include the immune serum marker O:7. The phage marker for this group is A54, as indicated in Table 6 below.

TABLE 6

Phage marker exclusive to the O:7(C1) antisera typed bacterial serovar group

Marker	Isolate	Quantity
A54	248,249,289	3

EXAMPLE 5

Production of fluorescent signal by positive phage markers

Bacterial saline suspensions were adjusted to 0.1 O. D. at A_{450} nm. The phage concentration employed ranged from an RTD value of 1 to 6 and depended on the specific phage or phage library used. One part of a bacterial suspension was mixed with one part of a phage suspension in 0.01 M phosphate buffer pH 7.7 including 0.2 % $CaCl_2 7 \cdot H_2O$. Detection proceeded as outlined above in Example 3. Results showed typical non linear reactivity. Close to 20 % of the cells were reactive to an 8 log phage concentration of the phage, and nearly 100 % of the cells were positive to an 8-9 log phage concentration (RTD of 5). Flow cytometry analysis of positive phage marked bacteria revealed that the fluorescence signal was not co-linear with the concentration of the phage employed (Figure 4).

EXAMPLE 6

Analysis of staphylococcus aureus and coagulase negative staphylococci by a novel phage library

A library of 600 phage markers was prepared for the diagnosis of *Staphylococci* bacteria isolated from bovine mastitis infected cows. A collection of the standard international propagating strain for *Staphylococci* which is composed of 25 type strains for coagulase positive bacteria (*Staphylococcus aureus)* and 15 type strains for

Staphylococci coagulase negative bacteria (*S. hycus, S. carnosus, S. chromogenes, S. xylosus*) was used as a reference. An API–STAPH test was performed for all bacteria (Biomeriue, France). API results are shown in Figures 5-6. The infective phages of each strain are presented in Figures 7, 8 and 9.

Figure 7 depicts the different isolates (serovars) of *Staphylococcus aureus* (API profile of 6736153) which were analyzed with a library including 600 phage markers (designated by numbers). 100 % threshold analysis of common phage markers shows 57 positive phages which are infective to all bacterial isolates. These phage markers are marked red in Figures 6 and 7. The phages which are infective to only a portion of the bacterial isolates are marked by black numbers.

Bacteria 44, 47, 127 and 125 (Figures 8) were also isolated from cows with mastitis. Bacteria 44 and 47 both have an API profile of 6736150, while bacteria 127 and 125 have an API profile of 6736152. The common markers for bacteria 127 and 125 were marked in blue while the rest of the markers are marked pink (Figure 8). Common markers from bacteria 47 and 44 (sharing a common API profile of 6736150) were marked green. Green and blue markers are presented by bacteria having API profiles of 6736150 and 6736152, indicating a possible relationship between bacteria having markers indicated herein by the blue, green and pink colors. The black and red marked marker groups are different and are present in bacteria having API profiles of 6736151 and 6736153.

Figure 9 presents non mastitis related *S. aureus* bacteria which show some cross reactivity with phages infective to bacteria presenting the red-black, blue-green-pink marker groups. PS-42E and PS-81 are bacteria used as propagating strains for phages that are a part of the standard phage typing set for *S. aureus* (originated from the international

center for phage typing in Colingdale, London, England). These bacteria have an API profile of 6736150. The low reactivity of these bacteria with the phage library prepared for the mastitis related *S. aureus* bacteria suggests a possible different origin. The PS-42E bacteria shows some relation to bacteria presenting the blue-green-pink markers, while the PS-81 bacteria, having an API profile similar to that of PS-42E, are cross reactive with phages associated with the black markers system. The PS-96 bacteria, an additional propagating strain, has an API profile of 6763130, is poorly marked, and shows no cross reactivity with phages associated with the above markers systems.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.